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A Breathing ATRP: Fully Oxygen Tolerant Polymerization Inspired by Aerobic Respiration of Cells

Alan E. Enciso†, Liye Fu†, Alan J. Russell and Krzysztof Matyjaszewski*

Abstract: The first well-controlled aqueous atom transfer radical polymerization (ATRP) conducted in the open air is reported. This air tolerant ATRP was enabled by the continuous conversion of oxygen to carbon dioxide catalyzed by glucose oxidase (GOx), in the presence of glucose and sodium pyruvate as sequential sacrificial substrates. Controlled polymerization using initiators for continuous activator regeneration (ICAR) ATRP of oligo(ethylene oxide) methyl ether methacrylate (OEOMA, M_n=500), yielded polymers with low dispersity ($1.09 \leqslant Đ \leqslant 1.29$) and molecular weights (MW) close to theoretical values in the presence of pyruvate. Without added pyruvates, lower MW were observed due to generation of new chains by H_2O_2 formed by reaction of O_2 with GOx. Successful chain extension of $POEOMA_{500}$ macroinitiator with $OEOMA_{300}$ confirmed a well-controlled polymerization ($Đ \leqslant 1.3$). The reactions in open air in larger scale (25 mL) were also successful. In addition, a "grafting from" polymerization was carried out from ATRP initiators covalently attached to Bovine Serum Albumin (BSA) with low amounts of catalyst (ppm vs. monomer) and short reaction time ($\leqslant$ 2 hours).

ATRP is one of the most widely used Reversible Deactivation Radical Polymerization (RDRP) techniques due to the large range of available monomers, initiators and catalysts that allow the synthesis of polymers with complex architectures and multiple functionalities.[1-4] The synthesis of polymers under aqueous conditions via ATRP was employed to grow polymers from biomacromolecules,[5-9] Although such polymerizations have been successful, one particularly vexing challenge is that ATRP is sensitive to oxygen and cannot be performed in open vessels. In the laboratory, ATRP is generally performed in a deoxygenated-capped container, or in the presence of excess reducing agents to remove limited amounts of air present in closed flasks.[10-12]

Oxygen is a diatomic molecule with two unpaired electrons in its outer shell that quickly capture radicals, forming relatively stable peroxy radicals, therefore stopping the polymerizations.[13, 14] Thus, oxygen is the undesired radical scavenger in radical polymerizations. To overcome this, deoxygenation with inert gasses, use of a glove box or other techniques such as "freeze, pump, thaw" are used for the effective removal of oxygen.[15]

Previously, Yagci[16] and Stevens[17-19] described the use of glucose oxidase (GOx) as an effective scavenger for oxygen in free radical polymerization and in the denoted enzyme-assisted reversible addition-fragmentation chain-transfer (RAFT) polymerizations, respectively. They also demonstrated the feasibility of polymerizations in open air in the presence of the enzyme, which has also been successfully carried out by other groups.[20, 21] However, the generated hydrogen peroxide (from glucose oxidase-catalysed step between oxygen and D-glucose), [22-24] was not removed and its fate has not been addressed.[25]

In an ATRP, hydrogen peroxide can oxidize Cu(I) activators and also it can initiate new chains via a Fenton-like reaction. Therefore, the enzyme-assisted deoxygenating strategy in ATRP is more challenging, as it should also include elimination of H_2O_2. Initially, we studied the GOx-catalyzed oxygen removal in an Initiators for Continuous Activator Regeneration Atom Transfer Radical Polymerization (ICAR ATRP) in aqueous systems[26] using 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044), glucose (200 mM), GOx (2 μM)[17] and NaBr (100 mM) in open air. Interestingly, the first attempts performed with stirring in completely opened vials did not yield any polymerization. However, in the absence of stirring, successful polymerizations were achieved. This indicated the importance of oxygen diffusion in the reaction, as previously reported.[18] Therefore, reactions in capped vials were carried out in order to avoid constant oxygen diffusion into the system, providing reproducible polymerizations, even with rapid stirring.

The initial polymerizations were conducted with a molar ratio of $[OEOMA_{500}]$/[Initiator]/[VA-044]/[Cu]/[TPMA]=200:1:0.3:0.2:0.4, using 20 vol.% monomer in 1X phosphate buffer saline (PBS) at 45°C (**Figure S1**). 2-Hydroxyethyl-bromoisobutyrate (HO-EBiB) was the initiator, yielding polymers with relatively low dispersity, ($Đ$~1.27). However, the number average molecular weights (M_n) were approximately 4 times lower than the theoretical values. This indicated formation of new chains, plausibly generated by H_2O_2. The use of α-Bromophenylacetic acid (BPAA), a more active initiator,[27] did not significantly improve control.

Additionally, different ratios of reactants were explored to enhance polymerization control. Experiments with different loadings of catalyst (100, 300, 500 and 1000 ppm) revealed that the amount of Cu can be reduced to 300 ppm; but at lower concentrations, the dispersity increased (**Figure S2**). Reducing the ratio of VA-044 to alkyl halide initiator (0.3, 0.1, 0.05, 0.02, and 0.01) slowed down the reaction and diminished control below the ratio 0.05 (**Figure S3**). Since the reactions were performed in 1X PBS, which contains 137 mM NaCl, no additional NaBr salt was added, as reported for other aqueous ATRP systems.[9] The addition of salt at concentrations higher than 100 mM slowed down the reaction (**Figure S4**).

When higher molecular weights were targeted, i.e degrees of polymerization (DP_n) >200, the obtained experimental MW, M_n = 68,000; which was nearly 5 times lower than $M_{n,Th}$ = 300,000, although all monomer was completely consumed (**Table 1, entries 1-5**). This suggested the generation of new chains from H_2O_2 produced by GOx, continued even in a closed vial. The formed H_2O_2 could further have reacted with Cu^I species via a Fenton-like reaction, yielding initiating radicals and new polymer chains[28-31] **(Scheme S2)**. To prevent formation of new chains, the reactive oxygen species should be eliminated and converted to

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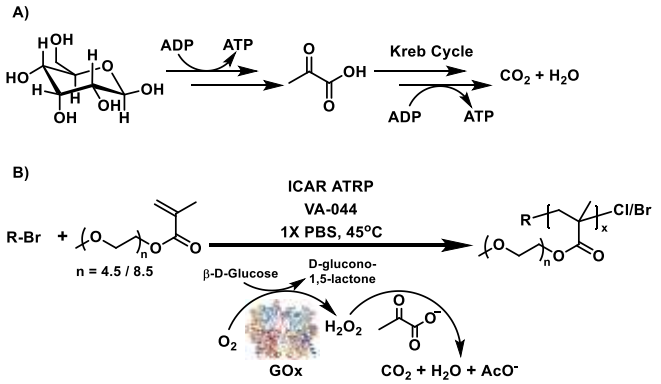
inert products that do not interfere with propagating radicals or catalyst.[32]

In some biological systems, such as in the aerobic respiration of cells, glucose and oxygen after several enzyme-catalyzed reactions produce CO_2 and ATP molecules. At the first stage, glucose proceeds through a series of transformations in the glycolysis cycle, which yields pyruvate and ATP, then pyruvate reacts with oxygen to generate acetyl-CoA that in the Krebs cycle forms CO_2 and additional ATP molecules (**Scheme 1A**).



Scheme 1. A) Aerobic respiration in cells. **B)** Breathing ATRP.

We decided to develop a bio-inspired polymerization system (**Scheme 1B**), in which GOx first catalyzed the conversion of glucose and oxygen into D-glucono-1,5-lactone and hydrogen peroxide and therefore eliminated oxygen inhibition of a radical polymerization. Then, in the second step, we reacted the toxic byproduct of oxygen removal, hydrogen peroxide, with sodium pyruvate (k = 3.5 × 10^5 M^{-1} sec^{-1})[33] yielding CO_2, acetate and water.[34] This green approach permits ATRP in open air by continuous elimination of oxygen without generation of new chains. The products did not interfere with propagating radicals or catalysts and do not generate new chains.

Experiments using the improved bio-deoxygenation conditions (**Table 1, entries 6-10**) for different targeted DP_n (100, 200, 400, 600 and 800) formed polymers with low dispersity (1.15 ≤ Đ ≤ 1.27) and reached quantitative conversions in less than 2 hours.

Chain extension was accomplished by either the direct addition of $OEOMA_{300}$ monomer (**Figure 2A**) to the vial after the first block reached ~95% conversion (monitored by NMR) or by stopping the reaction at 60% conversion, followed by dialysis in deionized water with a 5kD MW Cut-off membrane and subsequent lyophilization. The dry macroinitiator was redissolved to continue the polymerization of the second block (**Figure 2B**).

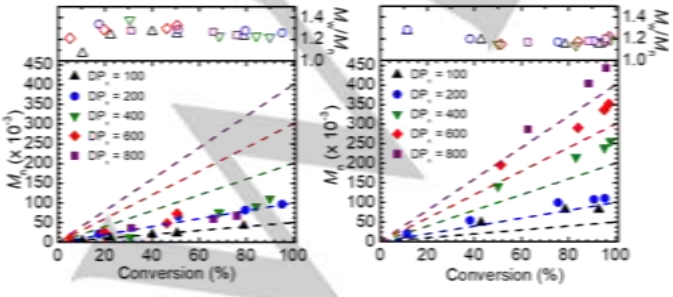


Figure 1. Corrected M_n (solid points), $M_{n,Th}$ (dashed lines) and dispersities (hollowed points) for ***Table 1*** (Left: entries 1-5; Right: entries 6-10).

Table 1. ICAR ATRP targeting various DP_n with 300 ppm Cu with (+) and without (-) 100 mM sodium pyruvate

Entry	M/I/VA-044/ Cu/TPMA	Sodium pyruvate	RXN Time (h)	Conv (%)	$M_{n,Th}$×10^{-3}	$M_{n,exp}$×10^{-3}	Đ
1	100/1/0.3/0.03/0.15	-	2	51	25.5	23.5	1.25
2	200/1/0.3/0.06/0.3	-	3	95	95.3	96.8	1.27
3	400/1/0.3/0.12/0.6	-	2	90	180	110	1.23
4	600/1/0.3/0.18/0.9	-	2	50	152	73.3	1.33
5	800/1/0.3/0.24/1.2	-	2	76	304	67.7	1.24
6	100/1/0.3/0.03/0.15	+	1.5	92	45.8	82.7	1.15
7	200/1/0.3/0.06/0.3	+	2	95	95.4	111	1.17
8	400/1/0.3/0.12/0.6	+	1.5	97	194	239	1.17
9	600/1/0.3/0.18/0.9	+	1.5	94	283	336	1.18
10	800/1/0.3/0.24/1.2	+	1.5	96	384	442	1.27

M = $OEOMA_{500}$, [M] = 10 vol % in 1X PBS, [NaBr] = 100 mM, [Glucose] = 200 mM, [GOx] = 2 μM, [Sodium Pyruvate] = 100 mM, T = 45°C.

This experiment confirmed the retention of chain end-functionality in the "breathing" ICAR ATRP.

Thermoregulation in this system (**Figure 2C**) was confirmed by temporal on/off experiments consisting on heating at 45 °C and cooling the system in iced water in 15 minutes intervals for 2 hours. VA-044 at ~44 °C has appropriate decomposition rate ($t_{1/2}$ = 10 h). Therefore, cooling the reaction in ice bath (0°C) essentially stopped the decomposition of VA-044 and generation of radicals, quenching polymerization and demonstrating possibility of temporal control. The final polymer displayed M_n matching the theoretical value and low dispersity (**Figure 2D**). Reactions were also carried out in open air in a 50 mL round bottom flask. They yielded polymers with dispersities and molecular weights identical to the model reactions (**Figure S5 and S6**), indicating good scalability.

To test the possibility of using this bio-inspired cascade system to modify biomolecules, the synthesis of protein-polymer hybrids was carried out by grafting from the surface of Bovine Serum Albumin (BSA). An ATRP initiator containing a cleavable ester moiety and anchoring N-hydroxysuccinimide (NHS) species was synthesized and covalently coupled to the accessible lysine residues on the surface of BSA in PBS.[9] The polymerization was

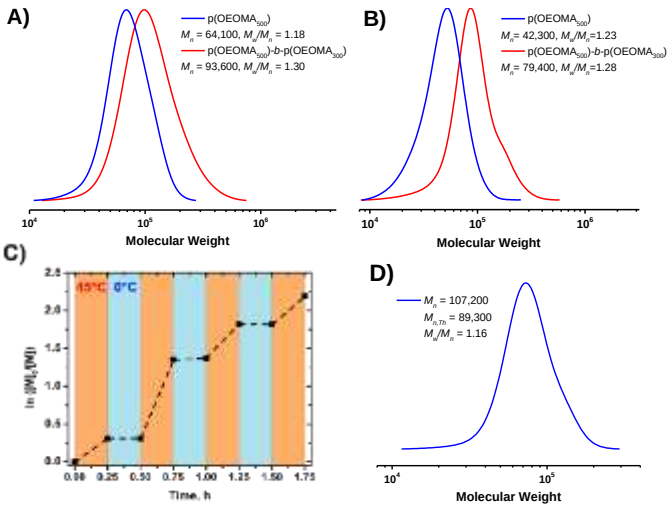


Figure 2: Chain extension experiments via **A)** One-pot strategy w/ pyruvate in open air; **B)** Purification-reinitiation strategy. **C)** Kinetic plots of temporal control of on-off study under different temperatures (45°C vs. ice bath) and **D)** GPC trace of final polymer product.

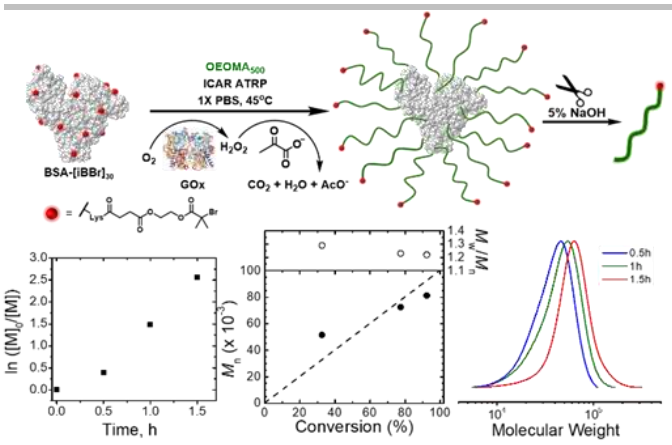


Figure 3. Modification of proteins with ATRP initiators and ICAR ATRP under open-air conditions.

proceeded under "breathing" ICAR ATRP conditions (**Figure 3**). After the polymerization of $OEOMA_{500}$ monomer was completed, the polymer was cleaved from the enzymatic structure under mild basic conditions (5% NaOH) and analyzed via THF GPC, confirming synthesis of well-defined polymers with predetermined molecular weight (M_n= 81,300) and low dispersity ($Đ$ = 1.22).

Model experiments were conducted to evaluate a reaction between H_2O_2 and Cu(I) species. In a sealed and degassed vessel, 1.25 μmol H_2O_2 was dissolved in 5 mL 1X PBS with or without sodium pyruvate (100 mM) and then 2 mM of CuBr/TPMA was added. Without pyruvate, a significant increase of absorption at 870 nm was observed by UV-vis spectroscopy, indicating formation of Cu(II)/TPMA complex ($\varepsilon_{[870]}$ = 222 $M^{-1}cm^{-1}$ in water, **Figure S7**). In the experiment with pyruvate, the Cu(II)/TPMA complex was not formed (**Figure S8**). Also, to mimic the polymerization conditions, the same concentrations of glucose and GOx with or without sodium pyruvate were dissolved in 3 mL of 1X PBS in a cuvette without degassing; then Cu(I)/TPMA stock solution was added. The results showed that the pyruvate successfully suppressed the oxidation of Cu(I), even in a flask open to air (**Figure S9**).

In conclusion, the first example of a fully oxygen tolerant well-controlled ATRP is reported. The aerobic respiration-inspired deoxygenation cascade with GOx and sodium pyruvate effectively eliminates generation of new chains by H_2O_2 in ICAR ATRP. The novel procedure was used for synthesis of well-defined block copolymers and protein-polymer hybrids.

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Conflict of interest

The authors declare no conflict of interest

Keywords: Oxygen • ATRP • Glucose Oxidase • Hydrogen Peroxide • Pyruvate

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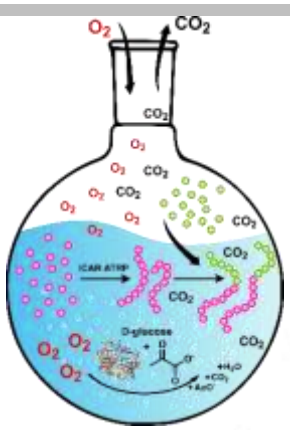

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